Clinical Potential of C- and P-MRS

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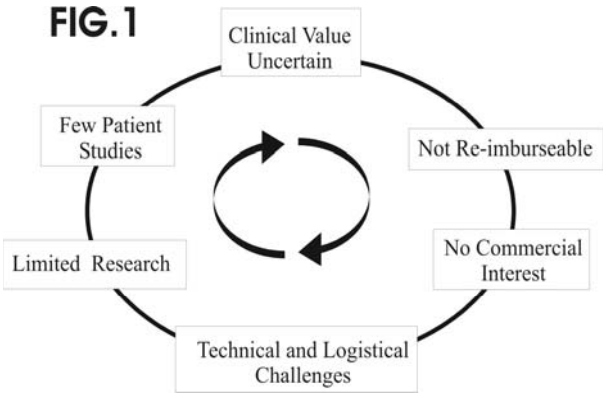
Introduction

Proton MRS is by far the most widely used *in vivo* spectroscopy technique in humans. This is due to the fact that standard MRI hardware components are used, making ^{1}H MRS widely available, that the concentrations of protons are high, and that the MR sensitivity of protons is higher then the sensitivity of other nuclei. On the other hand proton MRS is sometimes limited by its lack of specificity. For example, elevated total choline in ^{1}H MRS could be due to an increase of phosphocholine (PC), glycerophosphocholine (GPC), or free choline or any combination. Cr comprises both free creatine and phosphocreatine. ^{1}H MRS is also compromised by low spectral resolution and complex pattern of some metabolites due to homonuclear J-coupling. Finally, important metabolites such as ATP are not observed with ^{1}H MRS.

Challenges for ^{13}C and ^{31}P MRS



Apart from identifying relevant medical and biological questions, there are significant challenges for both ^{13}C and ^{31}P MRS. Both methods compete with MRI, the probably most powerful diagnostic imaging tool, for valuable time on expensive equipment. In addition, multinuclear spectroscopy of disease is challenging because of the need for the availability of these tools on clinical MR scanners. This requires additional hardware, including dual-tuned RF coils and proton-decoupling[a] capabilities which are not only expensive but sometimes, even if funds are available, hard to get installed on a clinical system. Both methods are also compromised by long acquisition times/low spatial resolution scans (due to low sensitivity)[b]. Finally, in particular for ^{13}C MRS, expertise with data processing which includes complex mathematical modeling is needed.

Not surprisingly multinuclear MRS finds itself in the difficult situation illustrated in **Fig. 1**. However, as discussed below, both modalities offer unique opportunities to examine tissue *in vivo*.

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[a]Proton-decoupling and the nuclear Overhauser effect (NOE) are essential in particular for direct detected ^{13}C MRS but also improve ^{31}P MRS considerably. It is not advised to attempt ^{13}C MRS without decoupling/NOE. Even the simplest application, such as measuring the lipid profile of a human leg would be quite demanding without the improved SNR and resolution facilitated by decoupling and NOE.

[b]An exception is ^{31}P MRS of skeletal muscle (e.g. leg). Due to the proximity of a surface coil to the leg muscle spectra of excellent quality can be obtained within a few seconds.

^{13}C MRS

Only a few groups have attempted ^{13}C MRS *in vivo*. The few studies undertaken illustrate the great promise of *in vivo* ^{13}C MRS and interest in ^{13}C MRS has grown considerably in recent years. A recent issue of *NMR in Biomedicine* (1) was exclusively dedicated to the application of ^{13}C MRS to study biological systems. The potential of ^{13}C arises from its biggest handicap: Low natural abundance of ^{13}C (^{13}C ≈1.1%, ^{12}C ≈ 98.9%) and the compromised sensitivity (≈ 1/50 of ^{1}H) renders ^{13}C MR spectroscopy *in vivo* very difficulty due to the inherently very low signal-to-noise ratio (SNR). This low natural abundance, on the other hand, is the key to new, exciting applications of *in vivo* MRS:

^{13}C MRS enables the investigation of metabolic pathways and the measurement of flux rates *in vivo* in humans after ^{13}C enriched substrate infusion.

Fig. 2 illustrates the steps involved for a typical ^{13}C MRS study after substrate infusion. *In vivo* ^{13}C studies of humans require large amounts of ^{13}C enriched substrates (expensive!), are technically and logistically challenging, and thus need to be planned carefully (2-4). Depending on the biological question asked, it needs to be decided what substrate should be infused for how long and in what fashion. For some applications oral administration may be appropriate (5-7) which would simplify the procedure considerably because one intervenous (i.v.) infusion line could be eliminated.

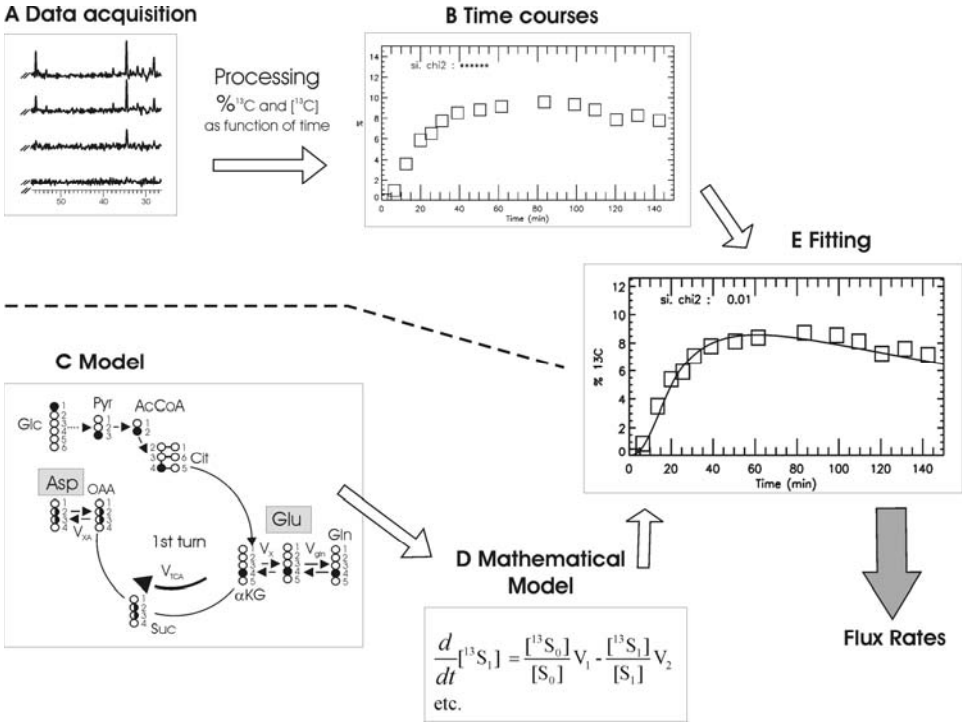

***FIG. 2**. (**A**) Dynamic ^{13}C MRS involves the sequential acquisition of ^{13}C spectra (typically every 2 – 10 minutes) after substrate infusion to monitor ^{13}C label accumulation. (**B**) The next step is the quantitation of spectra to generate time courses of ^{13}C concentration (or ^{13}C enrichment). (**C+D**) A set of differential equations is derived from the metabolic model. (**E**) By iteratively varying flux parameters (and pool concentrations) of the mathematical model to optimize the fit with experimental data, flux rates (and pool concentrations) can be determined.*

^{13}C enriched glucose has been used by several groups to study brain metabolism *in vivo*. Glucose is the principal substrate for energy metabolism for both neurons and glia cells in the brain and facilitates the *de novo* synthesis of many neurochemicals. Glucose is the first choice as substrate because of its rapid oxidation and the fast appearance of ^{13}C label in its metabolites and

its non-toxicity even at extremely high concentrations. Intravenously infused or orally administered glucose passes the blood-brain barrier and is readily metabolized through glycolysis and complete oxidation in the tricarboxylic acid (TCA)-cycle. ^{13}C enrichment of individual carbon atoms of glutamate (Glu), glutamine (Gln), aspartate (Asp), N-acetylaspartate (NAA), γ-amino butyric acid (GABA), lactate (Lac), alanine (Ala), and bicarbonate (HCO_3^-) follows and the *in vivo* rates of several of the principal bioenergetic pathways of the brain have been determined (2,3,8-11).

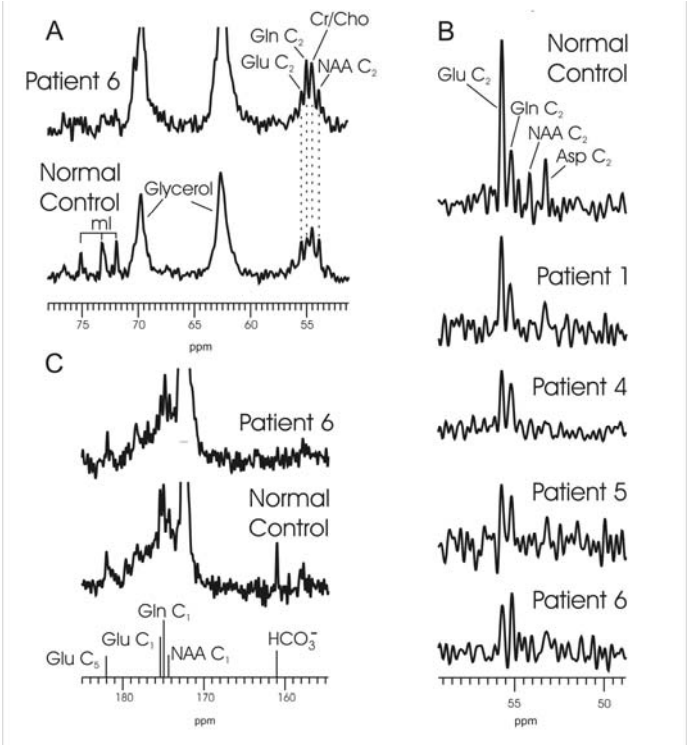

FIG. 3: a: *Natural-abundance ^{13}C MRS of a control and severe chronic hepatic encephalopathy (CHE). Myo-inositol (mI) was depleted, glutamine C_2 resonance was strikingly elevated (threefold) and glutamate was modestly reduced (20%).* ***b:*** *Difference spectra from a control and four patients representing increasing stages of CHE, calculated from acquisitions 60-100 min after start of infusion. A progressive reduction of glutamate C_2 labeling was observed.* ***c:*** *^{13}C spectra from the carbonyl region. At 60-100 min after start of infusion, ^{13}C incorporation into bicarbonate (the end product of complete glucose oxidation) is apparent in normal brain. In a severe case of grade III-IV CHE, bicarbonate enrichment was undetectable.*

Applications

Potential clinical applications include diseases associated with physiological and pathological alterations in cerebral glucose consumption. An example is hepatic encephalopathy where cerebral glucose metabolism is altered, in response to hyperammonemia (12,13). Indeed, striking abnormalities in glucose metabolism and glutamate and glutamine label accumulation were observed in patients with chronic hepatic encephalopathy which appeared to be more pronounced with increasing severity of the disease (**Fig. 3**) (11). Inherited mitochondrial diseases of the TCA-cycle or the electron transport chain also affect the overall rate of glucose oxidative metabolism in the brain and could be a target for ^{13}C MRS with glucose infusion. In addition to being the primary fuel of respiration, glucose is the source of α-glycerophosphate which is essential for biosynthesis of myelin phospholipids. ^{13}C MRS after ^{13}C enriched glucose infusion could therefore possibly contribute to understanding disorders in the developing brain including leukodystrophies and peroxisomal diseases which present as myelination defects on MRI. The information obtained from following the fate of ^{13}C labeled glucose goes beyond that of providing a rate for energy production. A tight coupling between cerebral glucose metabolism and glutamate neurotransmitter flux in humans has been proposed by Magistretti et al. (14). Aspartate, (a neurotransmitter?), can be studied *in vivo* in humans by its ^{13}C label accumulation. The role of NAA in mammalian brain, a neuronal/axonal marker which is central for its diagnostic power in ^{1}H MRS, is incompletely understood. NAA synthesis can be measured with ^{13}C MRS after glucose infusion in a clinical setting (5).

^{13}C labeled acetate: ^{13}C MRS studies employing ^{13}C enriched glucose as the substrate reflect predominantly the metabolism of neurons + glia. Acetate (Ac), an alternative fuel metabolized to acetyl-CoA only in the glial compartment (15) has been used in combination with ^{13}C glucose to separate glial from neuronal metabolism in cell preparations, tissue slices and *in vivo* in experimental animals (16,17). ^{13}C MRS after ^{13}C enriched acetate infusion therefore has the potential to investigate normal human glial metabolism and to elucidate brain diseases which originate in glia using the same MR technique as for glucose. Recent studies showed that the rate of Ac oxidation in human brain is ≈ 20% of the total neuronal/glial TCA-cycle rate in fasted human brain (18,19). In patients with epileptic seizures on ketogenic diet abnormal label accumulation in glutamate and glutamine was observed (20) (**Fig. 4**). These results are consistent with altered glutamate-glutamine neutrotransmitter cycling and adaptation to ketogenic diet with up-regulation of acetate oxidation relative to glucose oxidation.

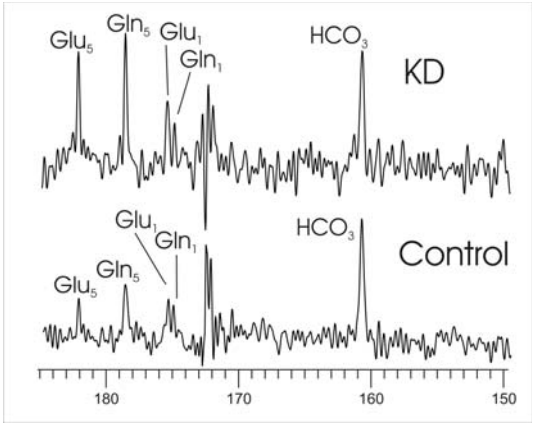

FIG. 4: Impact of ketogenic diet (KD) on astroglial acetate oxidation
(A) *^{13}C difference spectra obtained from ketogenic diet patients (average of three) and* ***(B)*** *controls after infusion of [1-^{13}C] acetate. ^{13}C label incorporation into Glu C_5 and Gln C_5 was more pronounced in patients. In addition, Glu C_1 and Gln C_1 enrichment was more prominent, whereas equivalent production of HCO_3 was observed.*

^{31}P MRS

Spectroscopy of living tissue started with ^{31}P MRS and it was not until technical advances in the early 1990s allowed efficient water suppression and reduction of eddy current artifacts that proton MRS become the dominant tool for *in vivo* spectroscopy. ^{31}P MRS allows the detection of phosphocreatine (**PCr**), **ATP**, and inorganic phosphate (**Pi**), and the measurement of **pH** from the chemical shift difference between PCr and Pi. ATP is the central provider of energy for all energy demanding processes in cells via the reaction:

$$ATP + H_2O \rightarrow ADP + Pi + energy \quad [1]$$

PCr functions as a battery and is used to maintain/replenish ATP pools via **creatine kinase** when demand exceeds mitochondrial ATP production during strenuous exercise:

$$PCr + ADP + H^+ \rightarrow ATP + Cr \quad [2]$$

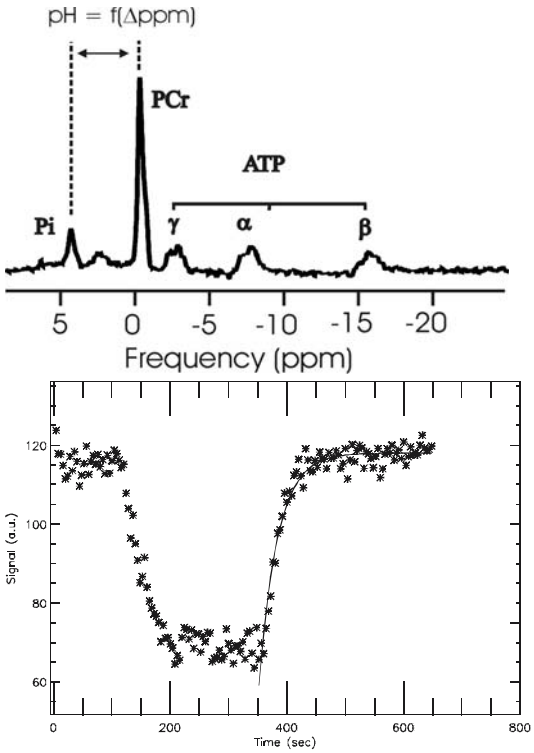

FIG. 5: *^{31}P MRS of skeletal muscle (top). Depletion and recovery of the muscle PCr levels during and after exercise (bottom).*

Finally, at rest ATP is restored via oxidative phosphorylation:

$$ADP + Pi + energy \rightarrow ATP + H_2O. \quad [3]$$

ATP can then be used to replenish PCr via creatine kinase:

$$PCr + ADP + H^+ \leftarrow ATP + Cr$$

While creatine kinase requires the uptake of one proton, anaerobic glycolysis generates a proton from lactate acid. From the simultaneous measurements of pH and PCr important information about glycolysis and creatine kinase can be obtained. PCr/Pi is an indicator for adequate mitochondrial oxidative phosphorylation. The resting ^{31}P spectrum of muscle, even in disease, is often unremarkable. Some form of exercise, while continuously scanning, is required to study energy metabolism. In particular **skeletal muscle** is readily accessible and can be studied rested and during exercise with a time resolution of a few seconds (**Fig. 5**). This makes ^{31}P MRS a superb tool to study energy metabolism and mitochondrial function non-invasively in skeletal muscle and to diagnose and quantify the extent of metabolic myopathies.

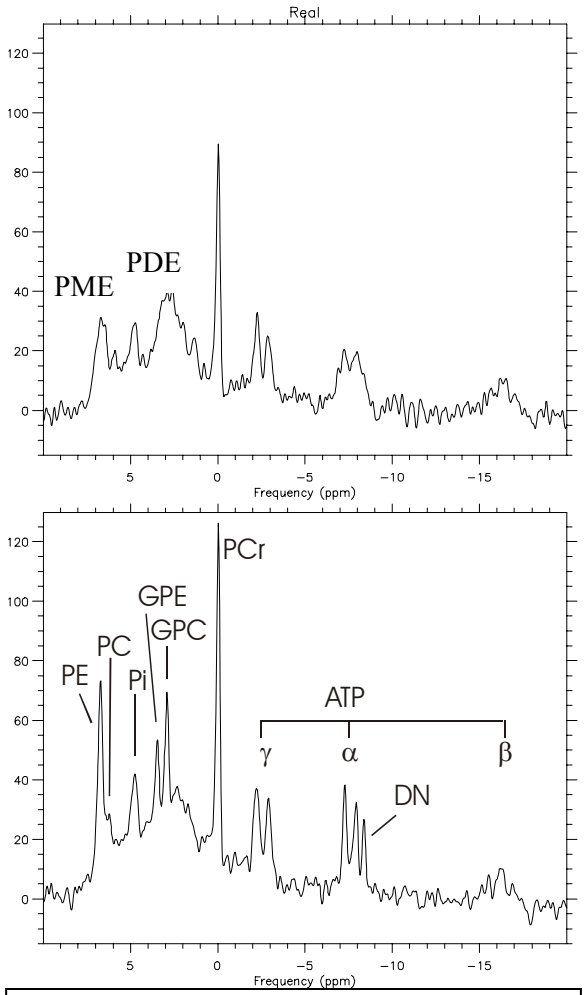

FIG. 6: *^{31}P (upper trace) and proton decoupled ^{31}P spectra of (normal) brain tissue. Proton decoupling is required to resolve peaks from PE, PC and GPE, GPC. By saturation of the protons a signal enhancement due to the Nuclear Overhauser Effect (NOE) is observed.*

^{31}P MRS of the diseased brain: A wide range of disease processes that include birth asphyxia, dementias, stroke, multiple sclerosis, epilepsy, mental disorders, hepatic encephalopathy, and tumors (21-39) were studied. The ^{31}P spectrum of **brain tissue** is more complex than spectra of muscle. In addition to ATP, PCr, and Pi, prominent phosphomonoester (PME) and phosphodiester (PDE) peaks are observed. **Proton-decoupling** is required to separate the PMEs phosphoethanolamine (PE), phosphocholine (PC) and the PDEs glycerophosphoethanolamine (GPE), glycerophosphocholine (GPC) from each other and from underlying broad signal from phospholipids (**Fig. 6**). These molecules are involved in myelin biosynthesis by methyl group metabolism and lipid transport and are components of a number of important biological compounds including the membrane phospholipids lecithin, sphingomyelin, and plasmalogen (40). Thus in the brain, ^{31}P MRS may provide useful information about (**i**) **energy metabolism** while it can be used at the same time to study diseases with (**ii**) **abnormal membrane metabolism**. In particular GPC and (possibly GPE) has been identified as a cerebral osmolyte (41,42) and ^{31}P MRS can be used to investigate disease which are believed to be associated with (**iii**) disturbances of **cerebral osmoregulation**. An example of the use of combined ^{1}H and

proton decoupled ^{31}P MRS to monitor the impact of treatment with hydrocortisone and testosterone of a subject with hypopituitarism is shown in **Fig. 7**.

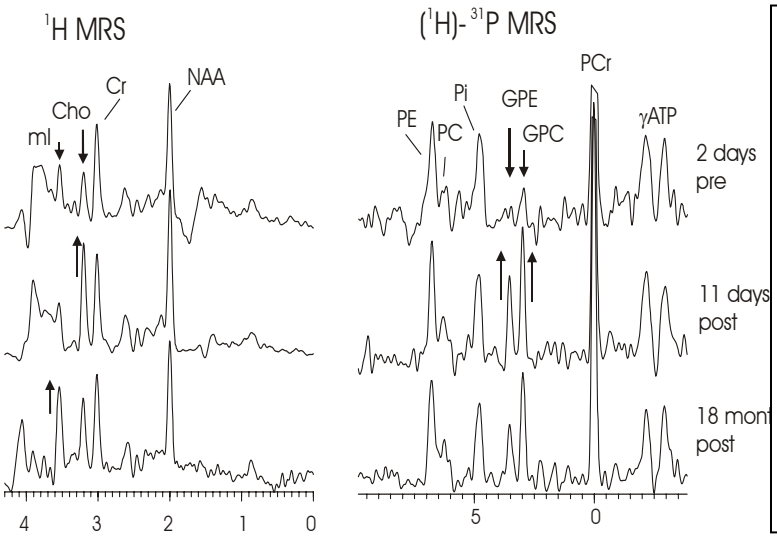

FIG.7: *Sequential ^{1}H and proton decoupled ^{31}P MRS in a 60 years old male with hyponatremia (NA = 113 mEq/l) due to hypopituitarism before and after treatment with hydrocortisone and testosterone. Before treatment, total choline was low (measured with ^{1}H MRS) and GPC as well as GPE were depleted. 11 days after treatment total choline and GPC and GPE were slightly above normal. 1 ½ years later total choline and GPC and GPE were within normal. Myo-inositol (mI) appeared prominent in the final ^{1}H spectrum. The significance and cause of elevated mI is uncertain. (PCr peak truncated).*

References

1. NMR Biomed 2003;16(6-7):301-457.
2. Mason GF, Gruetter R, Rothman DL, Behar KL, Shulman RG, Novotny EJ. Simultaneous determination of the rates of the TCA cycle, glucose utilization, alpha-ketoglutarate/glutamate exchange, and glutamine synthesis in human brain by NMR. J Cereb Blood Flow Metab 1995;15(1):12-25.
3. Gruetter R, Seaquist ER, Kim S, Ugurbil K. Localized in vivo 13C-NMR of glutamate metabolism in the human brain: initial results at 4 tesla. Developmental Neuroscience 1998;20(4-5):380-388.
4. Mason GF, Falk Petersen K, de Graaf RA, Kanamatsu T, Otsuki T, Shulman GI, Rothman DL. A comparison of (13)C NMR measurements of the rates of glutamine synthesis and the tricarboxylic acid cycle during oral and intravenous administration of [1-(13)C]glucose. Brain Res Brain Res Protoc 2003;10(3):181-190.
5. Moreno A, Ross BD, Bluml S. Direct determination of the N-acetyl-L-aspartate synthesis rate in the human brain by (13)C MRS and [1-(13)C]glucose infusion. J Neurochem 2001;77(1):347-350.
6. Moreno A, Bluml S, Hwang JH, Ross BD. Alternative 1-(13)C glucose infusion protocols for clinical (13)C MRS examinations of the brain. Magnetic Resonance in Medicine 2001;46(1):39-48.
7. Watanabe H, Umeda M, Ishihara Y, Okamoto K, Oshio K, Kanamatsu T, Tsukada Y. Human brain glucose metabolism mapping using multislice 2D (1)H-(13)C correlation HSQC spectroscopy. Magnetic Resonance in Medicine 2000;43(4):525-533.
8. Beckmann N, Turkalj I, Seelig J, Keller U. 13C NMR for the assessment of human brain glucose metabolism in vivo. Biochemistry 1991;30(26):6362-6366.
9. Pan JW, Stein DT, Telang F, Lee JH, Shen J, Brown P, Cline G, Mason GF, Shulman GI, Rothman DL, Hetherington HP. Spectroscopic imaging of glutamate C4 turnover in human brain. Magnetic Resonance in Medicine 2000;44(5):673-679.

10. Bluml S, Moreno A, Hwang J-H, Ross BD. 1-(13)C glucose magnetic resonance spectroscopy of pediatric and adult brain disorders. NMR in Biomedicine 2001;14(1):19-32.
11. Bluml S, Moreno-Torres A, Ross BD. [1-13C]glucose MRS in chronic hepatic encephalopathy in man. Magn Reson Med 2001;45(6):981-993.
12. Jessy J, Mans AM, DeJoseph MR, Hawkins RA. Hyperammonaemia causes many of the changes found after portacaval shunting. Biochemical Journal 1990;272(2):311-317.
13. Jessy J, DeJoseph MR, Hawkins RA. Hyperammonaemia depresses glucose consumption throughout the brain. Biochem J 1991;277 (Pt 3):693-696.
14. Magistretti PJ, Pellerin L, Rothman DL, Shulman RG. Energy on demand. Science 1999;283(5401):496-497.
15. Muir D, Berl S, Clarke DD. Acetate and fluoroacetate as possible markers for glial metabolism in vivo. Brain Research 1986;380(2):336-340.
16. Advances in Neurochemistry. In: Bachelard H, ed(s). editor. Volume Vol 8. New York: Plenum Press; 1997. p 1-413.
17. Cruz F, Scott SR, Barroso I, Santisteban P, Cerdan S. Ontogeny and cellular localization of the pyruvate recycling system in rat brain. Journal of Neurochemistry 1998;70(6):2613-2619.
18. Bluml S, Moreno-Torres A, Shic F, Nguy CH, Ross BD. Tricarboxylic acid cycle of glia in the in vivo human brain. NMR Biomed 2002;15(1):1-5.
19. Lebon V, Petersen KF, Cline GW, Shen J, Mason GF, Dufour S, Behar KL, Shulman GI, Rothman DL. Astroglial contribution to brain energy metabolism in humans revealed by 13C nuclear magnetic resonance spectroscopy: elucidation of the dominant pathway for neurotransmitter glutamate repletion and measurement of astrocytic oxidative metabolism. J Neurosci 2002;22(5):1523-1531.
20. Bluml S, Shic F, Lai L, Yahya K, Lin A, Ross BD. Glutamate-glutamine cycling in epileptic patients on ketogenic diets. In Proceedings, 10th International Society of Magnetic Resonance in Medicine 2002; Honolulu. p 417.
21. Cady EB, Costello AM, Dawson MJ, Delpy DT, Hope PL, Reynolds EO, Tofts PS, Wilkie DR. Non-invasive investigation of cerebral metabolism in newborn infants by phosphorus nuclear magnetic resonance spectroscopy. Lancet 1983;1(8333):1059-1062.
22. Azzopardi D, Wyatt JS, Cady EB, Delpy DT, Baudin J, Stewart AL, Hope PL, Hamilton PA, Reynolds EO. Prognosis of newborn infants with hypoxic-ischemic brain injury assessed by phosphorus magnetic resonance spectroscopy. Pediatr Res 1989;25(5):445-451.
23. Calabrese G, Deicken RF, Fein G, Merrin EL, Schoenfeld F, Weiner MW. 31Phosphorus magnetic resonance spectroscopy of the temporal lobes in schizophrenia. Biol Psychiatry 1992;32(1):26-32.
24. Laxer KD, Hubesch B, Sappey-Marinier D, Weiner MW. Increased pH and inorganic phosphate in temporal seizure foci demonstrated by [31P]MRS. Epilepsia 1992;33(4):618-623.
25. Hugg JW, Duijn JH, Matson GB, Maudsley AA, Tsuruda JS, Gelinas DF, Weiner MW. Elevated lactate and alkalosis in chronic human brain infarction observed by 1H and 31P MR spectroscopic imaging. J Cereb Blood Flow Metab 1992;12(5):734-744.

26. Younkin DP, Delivoria-Papadopoulos M, Maris J, Donlon E, Clancy R, Chance B. Cerebral metabolic effects of neonatal seizures measured with in vivo 31P NMR spectroscopy. Ann Neurol 1986;20(4):513-519.
27. Husted CA, Goodin DS, Hugg JW, Maudsley AA, Tsuruda JS, de Bie SH, Fein G, Matson GB, Weiner MW. Biochemical alterations in multiple sclerosis lesions and normal-appearing white matter detected by in vivo 31P and 1H spectroscopic imaging. Ann Neurol 1994;36(2):157-165.
28. Deicken RF, Calabrese G, Merrin EL, Meyerhoff DJ, Dillon WP, Weiner MW, Fein G. 31phosphorus magnetic resonance spectroscopy of the frontal and parietal lobes in chronic schizophrenia. Biol Psychiatry 1994;36(8):503-510.
29. Pettegrew JW, Keshavan MS, Minshew NJ. 31P nuclear magnetic resonance spectroscopy: neurodevelopment and schizophrenia. Schizophr Bull 1993;19(1):35-53.
30. Bluml S, Tan J, Harris K, Adatia N, Karme A, Sproull T, Ross B. Quantitative proton-decoupled 31P MRS of the schizophrenic brain in vivo. J Comput Assist Tomogr 1999;23(2):272-275.
31. Barbiroli B, Montagna P, Martinelli P, Lodi R, Iotti S, Cortelli P, Funicello R, Zaniol P. Defective brain energy metabolism shown by in vivo 31P MR spectroscopy in 28 patients with mitochondrial cytopathies. J Cereb Blood Flow Metab 1993;13(3):469-474.
32. Gonzalez RG, Guimaraes AR, Moore GJ, Crawley A, Cupples LA, Growdon JH. Quantitative in vivo 31P magnetic resonance spectroscopy of Alzheimer disease. Alzheimer Dis Assoc Disord 1996;10(1):46-52.
33. Albers MJ, Krieger MD, Gonzalez-Gomez I, Gilles FH, McComb JG, Nelson MD, Jr., Bluml S. Proton-decoupled (31)P MRS in untreated pediatric brain tumors. Magn Reson Med 2005;53(1):22-29.
34. Bluml S, Philippart M, Schiffmann R, Seymour K, Ross BD. Membrane Phospholipids and High-Energy Metabolites in Childhood Ataxia with CNS Hypomyelination. Neurology 2003;61:648-654.
35. Bluml S, Seymour KJ, Ross BD. Developmental changes in choline- and ethanolamine-containing compounds measured with proton-decoupled (31)P MRS in in vivo human brain. Magn Reson Med 1999;42(4):643-654.
36. Bluml S, Zuckerman E, Tan J, Ross BD. Proton-decoupled 31P magnetic resonance spectroscopy reveals osmotic and metabolic disturbances in human hepatic encephalopathy. Journal of Neurochemistry 1998;71(4):1564-1576.
37. Hoang TQ, Bluml S, Dubowitz DJ, Moats R, Kopyov O, Jacques D, Ross BD. Quantitative proton-decoupled 31P MRS and 1H MRS in the evaluation of Huntington's and Parkinson's diseases. Neurology 1998;50(4):1033-1040.
38. Tan J, Bluml S, Hoang T, Dubowitz D, Mevenkamp G, Ross B. Lack of effect of oral choline supplement on the concentrations of choline metabolites in human brain. Magnetic Resonance in Medicine 1998;39(6):1005-1010.
39. Negendank W, Li CW, Padavic-Shaller K, Murphy-Boesch J, Brown TR. Phospholipid metabolites in 1H-decoupled 31P MRS in vivo in human cancer: implications for experimental models and clinical studies. Anticancer Res 1996;16(3B):1539-1544.
40. Gillies RJ, Barry JA, Ross BD. In vitro and in vivo 13C and 31P NMR analyses of phosphocholine metabolism in rat glioma cells. Magn Reson Med 1994;32(3):310-318.
41. Lien YH, Shapiro JI, Chan L. Effects of hypernatremia on organic brain osmoles. J Clin Invest 1990;85(5):1427-1435.

42. Floegel U, Niendorf T, Serkowa N, Brand A, Henke J, Leibfritz D. Changes in organic solutes, volume, energy state, and metabolism asociated with osmotic stress in a glial cell line: a multinuclear NMR study. Neurochem Res 1995;20:793-820.